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A HEMORRHAGIC FEVER IN MONKEYS

[Following is the translation of an article by B. A. Lapin, S. N. Pokerman, L. A. Yakovleva, E. K. Dzhikidze, Z. V. Shevtsova, M. I. Kukseva, L. V. Danko, R. I. Krylova, Ye. Ya. Akbroyt, and V. Z. Agrba, Institute of Experimental Pathology and Therapy, AMN, USSR, Sukhumi, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology), No 2, 1967, pages 168-173. It was submitted on 12 May 1966.]

In the end of August 1964 it was noted that 2 rhesus monkeys out of 12 animals which were located in a small open-air cage in the northern sector had become sluggish and drowsy. Cramps developed periodically in one of them (around one year old). The monkeys were treated with antibiotics and general restoratives. In 2 days the animals died. By this time signs of illness appeared in the remaining 10 monkeys. Following a neurologic examination a diagnosis of encephalitis was made on the basis of such symptoms as drowsiness, sluggishness, ataxia, and tremors. Inspite of intensive treatment with antibiotics (penicillin and tetracycline group) all the monkeys died in 10 days. In 10 more days in a different sector (excursion department) which was located at a considerable distance from the first sluggishness and drowsiness were also noted in 2 monkeys. All 10 animals from this cage were transferred to an isolation ward, where in a period of 16 days they died with a clinical picture of disease similar to that described above.

In this same period the disease developed in 2 more sectors (south laboratory housing and Home No 6). Thus over a period of 2 weeks in 4 sectors of the nursery which were located at a considerable distance from each other and which were maintained by different personnel 28 monkeys became ill and died. The duration of illness and clinical and pathomorphological manifestations were similar in all the monkeys.

Subsequently new cases of the disease appeared in various sectors. Beginning with 12 October a new wave of disease developed in the northern sector in the laboratory housing. It gradually increased and on the 7th day, having reached a maximum, started to decline. The last case of the disease was recorded on 28 Oct.

Among monkeys located in the southern laboratory housing and Home No 6 new cases of the disease were also observed. One monkey became ill in a new place - Home No 2.

On 17 October a strict quarantine was placed on the Institute and there was a complete cessation of all experimental work with animals. The transfer of animals from group to group was halted.

Only persons who were taking direct care of the monkeys were permitted in the monkey quarters. Personnel and inventory for each sector were securely fixed. Disinfection, disinsectization, and deratization were carried out in all the premises of the nursery.

All told during the period of the outbreak 62 monkeys became ill and all of them died. It is necessary to note that only animals which were located in accommodations became ill. Among monkeys living in cages outdoors not one case of illness was recorded. Only Macacus monkeys became ill: rhesus, lapunder, Assam, and brown. Among the sick there were monkeys of various age and sex; some had lived in the nursery for a long time and others had arrived 3-4 months prior to the outbreak of the disease from countries of Southeastern Asia.

As already indicated the first clinical symptoms of illness were sluggishness, drowsiness, and rejection of food. During the first 2-3 days the stated manifestations increased and added to them were ataxia and tremors of the head and limbs. In some of the monkeys there was noticeable anisocoria, ptosis of the eyelids, rigidity of the occiput, a lowering of muscle tone and tendon reflexes, especially of the rear extremities, spontaneous urination, and edema of the snout. Following touch stimulations of the solos clonic spasms developed. In some monkeys these turned into epileptoid seizures. Beginning with the 5th day of the disease symptoms of hemorrhagic diathesis appeared: hemorrhage on various sectors of the skin, bleeding from the gums, nose, and intestines. Blood coagulation was sharply lowered (bleeding time was 12-35 minutes with a norm of 1-2 minutes). The disease was accompanied by an increase of body temperature up to 40° for 1-2 days with a sharp drop before the death of the animal. Overall duration of the disease fluctuated from 5 to 14 days.

Shifts in the blood-forming system were very characteristic. In the first 2-3 days following the onset of the disease in half of the monkeys the number of leukocytes fluctuated from 3,050 to 10,200, and in the remaining animals the level of leukocytes was high - from 12,000 to 22,000. On the 6-8th day the number of leukocytes increased noticeable (30,000--40,000). On the part of the red blood on the 2-5th day of illness in some of the monkeys erythrocytosis was noted (8-10 million) with a subsequent lowering to initial values and lower. In this period there was also a lowering in the amount of hemoglobin (down to 45%). At the climax of the disease the ESR was speeded up to 60-70 mm/h. Also noted were neutrophilosis with a shift to the left down to individual myelocytes and immature ones, lymphopenia, aneosinophilia, monopenia, anisocytosis of thrombocytes, and the appearance in the peripheral blood of reticulo-endothelial, plasma cells, erythro- and normoblasts, often in microform. Attention is merited by the presence of microforms of neutrophiles with pycnotypical cells, the appearance of erythrocytes with basophilic granularity, their polychromatophylic nature and anisopoikilocytosis.

In the marrow the number of young cells of a neutrophilic nature was increased up to 57-60% with a simultaneous decrease in cells of a red nature.

The appearance in the peripheral blood of microforms of neutrophiles and erythro- and normoblasts with the simultaneous detection of reticulo-endothelial and plasma cells was an early diagnostic sign.

The combination of changes during pathologic-anatomical examinations makes it possible to reliably identify the given disease. One of its most constant manifestations is the development of hemorrhagic diathesis. Following autopsy of the dead animals hemorrhage was not revealed in only 14 (with a duration of illness of 3 up to 15 days). In the majority of cases hemorrhagic diathesis did not have a severe nature and was manifested by punctulate hemorrhages in various organs. In more than half of the cases hemorrhages were detected in the mucous membrane of the gastro-intestinal tract. It is necessary to note the lesions of the duodenum. Though this was not detected in all of the animals in a third of the cases the hemorrhages were exceedingly numerous. Often a diffuse hemorrhagic impregnation of the wall was noted in the duodenum. Here the inner surface of the intestine was reddish purple and the wall of the intestine was sharply edematous. Microscopically scattered necrosis was revealed. It encompassed the mucous membrane and the lining sections of the intestinal wall. With approximately the same frequency changes were revealed in the mucous membrane of the large intestine and considerably less often in the jejenum and the ileum. They differed from changes in the duodenum by a considerably lesser degree of evidence and prevalence, characterized by the development of a nonserious catarrh with a soft hemorrhagic component. Based on the frequency of the development of hemorrhage in it the heart is the second organ. By macroscopic investigation during autopsy hemorrhages were detected in the heart (more often in the epicardium and endocardium and less often in the myocardium) of half of the monkeys. However, histologically hemorrhages were observed in the heart in almost all the cases. Approximately as often as in the heart hemorrhages were observed macroscopically in the lungs. Less often (in a third of the cases) hemorrhages were determined in the brain membrane, and even less often - in the skin and tissue of the brain. Numerous hemorrhages were encountered variably in all the internal organs.

In many organs and tissues the vessels were sharply plethoric and stases were detected often. In the vascular wall plasmorrhagia was traced, and there was infiltration of the vascular wall by histocytic and leukocytic elements. Plasmorrhagia was encountered most often in the spleen and lungs. Infiltration of the vascular wall was detected most often in the lungs and was traced regularly in the central nervous system. In brain tissue (with the exception the membranes) perivascular infiltration often bore a glial nature, and not mesenchymal. Vascular endothelium was partially subjected to death and peeling. Thrombus was disposed in places in sectors of

infiltration in the vessel.

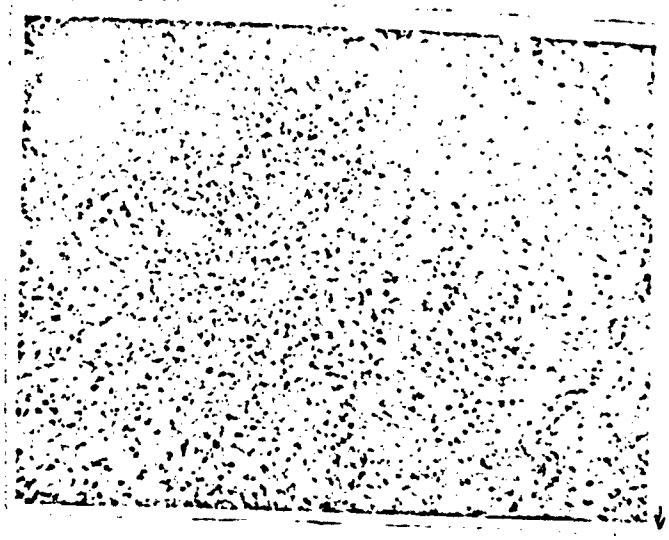

Fig. 1. Death of cells in follicles of the spleen. 3rd day of illness. Staining with hematoxylin-eosin. Ob. x 10, gomal' [2] 6.

The change in the spleen was typical. Its enlargement (often very significant - 4-5 times) was observed in dead animals in almost all the cases. Only in individual animals was the spleen enlarged following autopsy. The spleen was dense with rounded edges and a taut smooth capsule. In a section the pulp was dark red, did not protrude, and did not yield scrapings. As a rule on the background of the pulp it was possible to distinguish numerous small follicles surrounded by a dark red ring of hemorrhages. Histologically the enlargement of the organ was connected with its sharp hyperemia and perspiration of protein substances into the intersinus septa. The sinuses were empty and collapsed. Attention was drawn to the sharp reduction in the number of cells in the white and red pulp of the organ and the appearance of a large amount of lipids. Follicles for the most part were reduced. In light centers the diffuse disintegration of cells was constantly traced, (Fig. 1). Along the periphery of the follicles, less often in other sectors of the spleen, hemorrhages were frequently encountered. Similar changes could have been observed regularly in various groups of lymph nodes.

Just as regular and typical as the changes in the spleen was the involvement in the process of the central nervous system, particularly the brain. First of all it was manifested in the sharpest hyperemia of membranes and brain tissue right up to the development of stases and the appearance of numerous, primarily perivascular, hemorrhages. Hyperemia was often accompanied by the emigration of nuclear elements of the blood from the vessels (Fig. 2), primarily

of segmentonuclear leukocytes. In certain cases perivascular infiltration was expressed weakly. In some of the cases the copious emigration of cells into the membranes gave the process the nature of meningitis, and massive leukocytic connections were detected in the brain tissue. Leukocytic infiltration was particularly profuse during the accumulation of microbes in the lumina of the vessels.

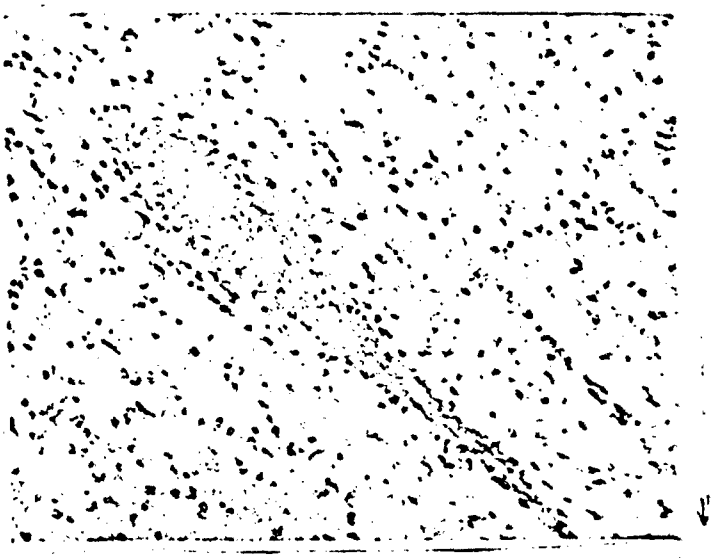

Fig. 2. Perivascular infiltration in the brain. 6th day of illness. Stained with thionine. Ob. x 40, gomal' /?/ 6

In cases with nonexpressed microbism in the brain tissue there was a predominance of glial perivascular connections. Damage to nerve cells was scattered but did not have a serious nature. It was expressed in the swelling of bodies and neuron processes which was accompanied by the pulverization of a gummy /nisslevskoye/ substance. Less often hyperchromatosis of nuclei and vascuolization of the protoplasm were observed. Individual cells perished with manifestations of hyperchromatosis and cirrhosis or, on the contrary, were subjected to lysis with the formation of cellular shadows. The stated changes were observed most often among the motor cells of the forward central convolution, in nuclei of the hypothalamus, the motor nucleus of the trigeminal nerve and nuclei of vestibular nerves, nuclei of the cerebellum, Purkinje cells, and also in cells of the commissure of the trunk portion of the brain. In some of the cases swelling of nerve cell processes with their profuse coloration was detected almost everywhere in the brain and spinal cord. Vacuolized and also pycnomorphic cells could be encountered primarily close to vessels with infiltrated walls.

Phenomena of dystrophy were observed regularly in parenchymatous organs. Constantly in all the dead animals accumulations of microbes were determined in the capillaries and small vessels of internal organs.

Out of the 62 sick monkeys 33 were subjected to intra-vital and postmortem bacteriological investigation. Blood cultures, made 4-8 days prior to death, were sterile in the majority of cases. Over a period of 1-3 days prior to death diverse microbes were sown from the blood in the majority of animals. These were primarily staphylococcus and coliform bacteria, and also enterococcus, pneumococcus, and B. perfringens. During the investigation of material from dead monkeys the degree of insemination of organs with microbes in the majority of cases was intensive. The greatest number of microbes was detected in the kidneys, cerebellum, liver, and spleen. This was determined both during inoculation on artificial nutrient media and during examination of smear imprints. Only in rare cases the material taken from the dead monkeys turned out to be sterile.

Assuming the possibility of a viral etiology for the given disease, rhesus monkeys were experimentally inoculated with filtrates of brain tissue (the material was filtered through Chamberland filters - L_5, L_7, L_{11}). The disease with the typical clinical and pathologo-anatomical picture was reproduced regularly in this species of animal. The urine and blood also turned out to be infected. Attempts at infecting other species of monkeys and small laboratory animals were not successful.

Discussion

Among rhesus monkeys at the Sukhumi monkey farm an outbreak of a disease was observed. The infectious nature of this disease did not cause any doubt. The main clinical manifestations of the disease are a brief fever, progressive muscular weakness, somnolence, tremors of the head and extremities, ataxia, hemorrhagic diathesis, leukopenia, alternated with leukocytosis, and unique morphological changes in the peripheral blood. Apparently in 100% of the cases the disease terminated in death. The feasibility of experimental reproduction of the disease on monkeys following their infection with filtrates from sick animals testifies to the viral nature of this disease.

A thorough epidemiological analysis showed that the source for spreading the disease was a group of Indian rhesus monkeys which came to the monkey house on 11 Aug 1964. Out of 60 monkeys in this group 55 died in the course of a month. The disease was not identified in time and the cause of death was diagnosed as various infectious diseases which are usually encountered in monkeys during their acclimatization period. Only the retrospective analysis of data from the pathomorphological investigation made it possible to establish that in at least 25% of the cases the described fever took place without a doubt.

Apparently at first the infection was transmitted from the newly arrived monkeys to monkeys at the farm by personnel who took care of both groups of animals. It is doubtless that in some of the cases the infection was spread during the manipulation of blood which was taken for laboratory investigation because of an inadequate sterilization of instruments. For example, out of 11 rhesus monkeys which were kept in 6 cages located on different levels of the same building, and from which blood was taken from the ear frequently by means of scarification with the same scapel, 9 animals became sick in the course of 5 days. An analogous situation was observed following the prophylactic injections of antibiotics and gamma-globulin on monkeys which were kept separate, when in a number of cases the healthy monkeys were handled after the sick monkeys were treated. Thus the infectious onset could be attributed to blood. This was also confirmed experimentally.

The infection was also spread by the close contact of animals which were kept in the same cage. The source of infection apparently was urine, since it contains active infectious material. The possibility of transmitting the infectious beginning by the aerial-droplet path is less probable. This is testified to by the nature of the outbreak and the absence of disease among monkeys who were separated from the sick ones only by a screen. It is unknown how the disease is transmitted and spread under natural conditions. It can only be assumed that in the jungles blood-sucking insects play a leading role in the transmission of the infection.

Our materials, accumulated in over 39 years of existence of the Sukhumi monkey farm, and also data from the literature [1-5], testify to the fact that this was the first time that such a disease was encountered in monkeys in captivity. Clinical, hematological, and morphological manifestations of the disease along with certain epidemiological facts give us a basis to propose that the disease belongs to the group of hemorrhagic fevers of viral etiology. In India a similar disease of monkeys is known. It is called the Kyasanur forest disease [6]. Its etiological factor is a virus which is close to the virus of tick-borne spring-summer encephalitis. However, the disease observed by us differs clinically and morphologically from Kyasanur forest disease. In our cases the central nervous system was involved regularly and damage to vessels was observed. In addition to this, in contrast to Kyasanur forest disease this disease was not reproduced in one species of laboratory-animals with the exception of the rhesus monkey.

We propose that the disease observed by us is a new nosological form, brought into the Sukhumi monkey farm from India.

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